

A FLUORESCENCE STUDY OF INTERACTION BETWEEN HUMAN CENTRIN 2 AND A PEPTIDE FROM THE PROTEIN XPC

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REZUMAT

Obiectiv: Centrina umană 2 este o proteină membră a superfamiliei proteinelor cu motivul mâinii de tip EF (EF-hand) cu un rol important în duplicarea și separarea centrozomilor în timpul diviziunii celulare. Această proteină are un posibil rol important în procesul de reparare a acizilor nucleici. Disfuncționalitatea acestui proces determină dereglări severe la om, ca boala ereditară numită xeroderma pigmentosum (XP), caracterizată prin fotosensibilitate ridicată și o incidență mare a cancerului indus de lumina solară. Scopul acestei lucrări este de a evalua interacțiunea dintre această proteină și peptidul P1-XPC.

Material și metode: Interacțiunea acestei proteine cu peptidul P1-XPC derivat din proteina XPC a fost studiată prin fluorescența de emisie. S-a utilizat spectrofluorimetrul Jasco F-777.

Rezultate: Rezultatele arată că P1-XPC formează, în prezența ionilor de calciu un complex 1:1 cu centrina umană 2, cu o constantă de afinitate $K_a=6.3 \cdot 10^7 M^{-1}$.

Concluzii: Între centrina umană 2 și peptidul P1-XPC există o interacțiune care implică modificări conformaționale, triptofanul din peptid fiind puternic ascuns într-o cavitate hidrofobă a proteinei.

Cuvinte cheie: centrina umană 2, peptid P₁XPC, fluorescență de emisie

ABSTRACT

Objective: Human centrin 2 is a protein belonging to the superfamily proteins with EF-hand motif. It has an important role in centrosome duplication and separation during the cell cycle. The protein has a possible major role in the process of nucleotide excision repair. Its dysfunction determines severe disorders in humans such as xeroderma pigmentosum, a hereditary disease characterized by a high photosensitivity and a large incidence of sunlight induced cancer. The aim of this fluorescence study is to evaluate interaction between this protein and peptide P1-XPC (XPC complex - which plays a key role in the initial phase of nucleotide excision repair (NER) process).

Material and Methods: Interaction between HsCen2 and P1-XPC from the protein XPC (a molecular component involved in DNA repair) was studied using fluorescence spectroscopy. Fluorescence was measured using a Jasco F-777 Spectrofluorimeter.

Results: The results show the peptide binds with high affinity ($K_a=6.3 \cdot 10^7 M^{-1}$) and 1:1 stoichiometry to human centrin 2.

Conclusions: There is an interaction between the HsCen2 with P1-XPC and this interaction involves a deep embending of the tryptophan residue into the hydrophobic pocket created by the EF-hand domain.

Key Words: human centrin 2, peptide P1-XPC, tryptophan fluorescence

INTRODUCTION

The proteins are the most complex organic substances with different roles in living organisms. It is very important to characterize the biological functions of the biomolecules.

One of the current techniques used in molecular biophysics for studying the structure and the dynamics

of proteins is the fluorescence spectroscopy. This technique has become very popular because of its sensitivity to changes in the structural and dynamic properties and because it requires a very small amount of substance. Human centrin 2 (HsCen2) was first discovered in the distal lumen of centrioles where its presence is required for normal centriole duplication during the cell cycle. The centrin is usually found in association with the microtubule organizing centres (centrosome in animal cells, and spindle pole bodies in yeast) - that are cytoplasmatic organelles, with an important role in the microtubules structural and temporal organization.¹ A large fraction of the cellular centrin is not permanently associated with the centrosome, but it fractionates with the cytoplasm and nuclei in human cells.² Recently, studies conducted in the Hanaoka group shed a new light on the possible

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role of nuclear HsCen2 fraction in nucleotide excision repair (NER) process - process which is a major pathway for recognition and removal of bulky DNA lesions.³ Its dysfunction produces severe disorders in humans such as xeroderma pigmentosum, a hereditary disease characterized by a high photosensitivity and a large incidence of sunlight induced cancer. One of the molecular components involved in this DNA repair the XPC complex - which plays a key role in the initial phase of NER and is involved in the recognition of the DNA damage.

As the peptide P1-XPC contains a tryptophan residue, the fluorescence properties of the peptide were used to obtain complementary data about the protein/peptide interaction.

MATERIAL AND METHODS

The studied protein was HsCen2, having two domains, each containing two putative EF-hand motifs for Ca²⁺ binding. Its C-terminal domain is highly disordered in the apo state but becomes better structured in the presence of Ca²⁺.⁵

Interaction between HsCen2 and the peptide P1-XPC was monitored by fluorescence spectroscopy. Fluorescence was measured using a Jasco FP-777 Spectrofluorimeter. The titration was performed by addition of the protein HsCen2 (final concentration 3 mM) to the peptide P1-XPC solution in MOPS buffer (3-(N-morpholino) propane sulfonic acid), 50 mM MOPS (pH=7.4), 20 mM KCl, 1 mM CaCl₂ with and without calcium. To estimate the equilibrium binding constant of peptide to HsCen2 the following conditions were used: the binding equation of peptide P1-XPC to human centrin 2 is:²



Where P is protein, L is ligand and PL is complex.

The affinity constant of P1-XPC/HsCen2 results from this equation:

$$k_a = \frac{[pl]}{[p][l]} \quad [2]$$

Where (PL) is complex concentration, [P] is concentration of free protein and [L] is concentration of free ligand.

The equation used for fitting curve from figure 2 is:

$$y = \frac{1}{2K_a} \left\{ (K_a x + aK_a + 1) - \sqrt{(K_a x + aK_a + 1)^2 - 4aK_a^2 x} \right\} \quad [3]$$

Where y = (pl) and x =(p) + (pl)

RESULTS

Solutions of the free peptides containing tryptophan residue have a maximum fluorescence emission at 350 nm. The binding of peptide P1-XPC to HsCen2 affects the fluorescence of tryptophan residue and the maximum of the fluorescence emission is blue shifted (320 nm). (Fig. 1)

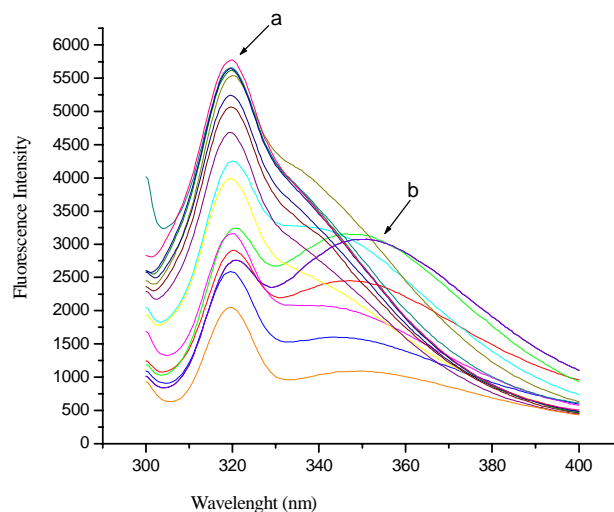


Figure 1. Fluorescence enhancement of P1-XPC (0.2 μM) with HsCen2, in 50 mM MOPS (pH=7.4) 20 mM KCl, 1 mM CaCl₂ using high sensitivity

The arrows in the Figure 1 have the following meanings: a) the maximum of the free tryptophan, b) the maximum of the tryptophan in the P1-XPC/HsCen2 complex. The absorption of the tryptophan is at 290 nm but the maxima in the figure 1 appear at 350 nm, respective at 320 nm and it is an illustration of the Stokes band.

The affinity constant of peptide P1-XPC for HsCen2 was estimated from the maximum of fluorescence emission of tryptophan in the complex P1-XPC/HsCen2, plotted as a function of HsCen2 concentration, in presence of calcium Ca²⁺ (Fig. 2). Fitting the data to equation 3 gives the constant is K_a = 6.3*10⁷M⁻¹.

The binding properties of HsCen2 were also studied in absence of calcium Ca²⁺ and the buffer conditions were: 50 mM MOPS (pH=7.4), 20 mM KCl, 1 mM EDTA.

Minor changes in fluorescence emission of complex P1-XPC/HsCen2 in presence of EDTA suggest that in these conditions the binding is much weaker.

DISCUSSION

Using calorimetry experiments, it was recently identified a fragment of 17 residues (P1-XPC) of XPC

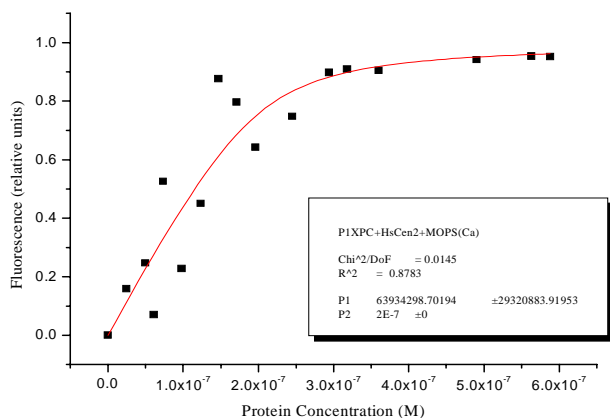


Figure 2. Maximum of fluorescence intensity of P1-XPC (0.2 μ M) with HsCen2 in 50 mM MOPS (pH=7.4) 20 mM KCl, 1 mM CaCl₂ versus HsCen2 concentrations

protein that binds with high affinity to HsCen2. The peptide binding is Ca²⁺-dependent with a stoichiometry 1:1.⁴

Structural NMR studies also provided evidence that HsCen2 binds calcium with a high affinity ($\sim 10^5$ M⁻¹) and the C-terminal domain of protein is fold into conformation with a large exposed hydrophobic area.⁵ This conformation allows strong binding of peptide P1-XPC to HsCen2 which was showed here also by using fluorescence spectroscopy.

CONCLUSIONS

When the peptide P1-XPC binds to the HsCen2 we noticed a blue shift in the emission maxima. This means that the polarity of the tryptophan environment is highly decreased. This also means that there is an interaction between the HsCen2 with P1-XPC and this interaction involves a deep embedding of the tryptophan residue into the hydrophobic pocket created by the EF-hand domain.

In absence of the calcium ions in the buffer solution, the emission spectra do not change significantly and this means that the apo-proteins is not able to bind to P1-XPC. Therefore the P1-XPC-HsCen2 binding is calcium dependent.

The results presented here are in good agreement with previous results obtained by calorimetry studies that revealed the high affinity of the P1-XPC for the HsCen2.

These results emphasize the fact that human centrin 2 has a role in sunlight induced cancer. Information about the formed complex HsCen2/P1-XPC help to find out the adequate investigation and treatment ways of this disease.

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